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4TH STAGE
TOXICOLOGY

LAB. 3

CALCULATION OF LD50

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Background

- **Toxicity test examine toxic effects when a chemical is absorbed into the body, via mouth, skin, lungs.**

- **The most common test of acute (short-term) toxicity is the LD50 test.**

- **Many different substances are tested in this way, including all drugs, agricultural chemicals, cleaners, some cosmetics and their ingredients.**

Definition of LD50

- **LD stands for "Lethal Dose".**
- LD50 is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals.
- The smaller the LD50 value, the more toxic is chemical. The opposite is also true: the larger the LD50 value, the lower the toxicity.

50% of the population dies

50% of the population lives

LD50 toxicity definition
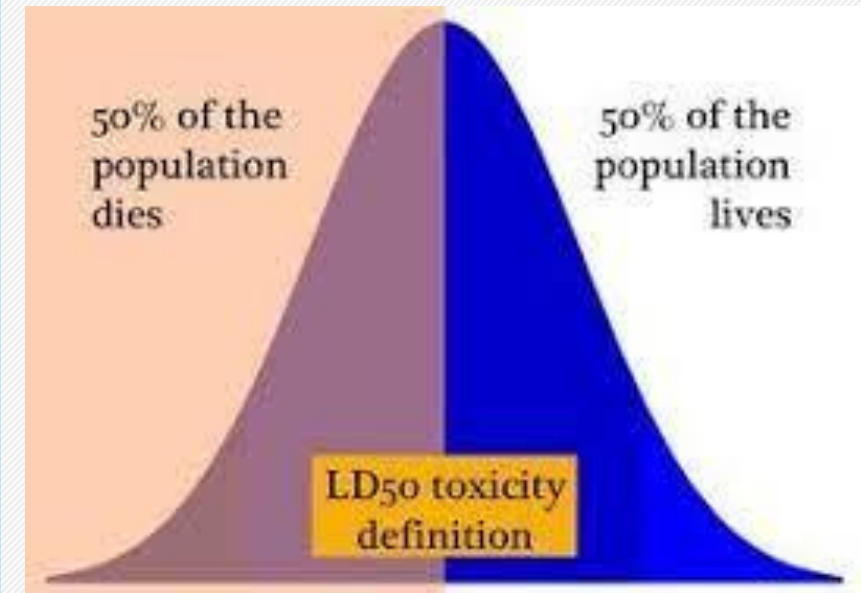
Animals and Routs

- Mice, rats, rabbits, guinea pigs, cats, dogs, fish, monkeys and birds are use for LD50 study.

- The LD50 values of a new drug are determined by various route of administration such as the intravenous, intraperitoneal, subcutaneous, and oral routes.

Animals and Routs

- **Example, some LD50s (with different rout of exposure) for Dichlorvos, an insecticide commonly used in household pesticide strips: -**
- Oral LD50 (rat): 56 mg/kg
- Dermal LD50 (rat): 75 mg/kg
- Intraperitoneal LD50: (rat) 15 mg/kg
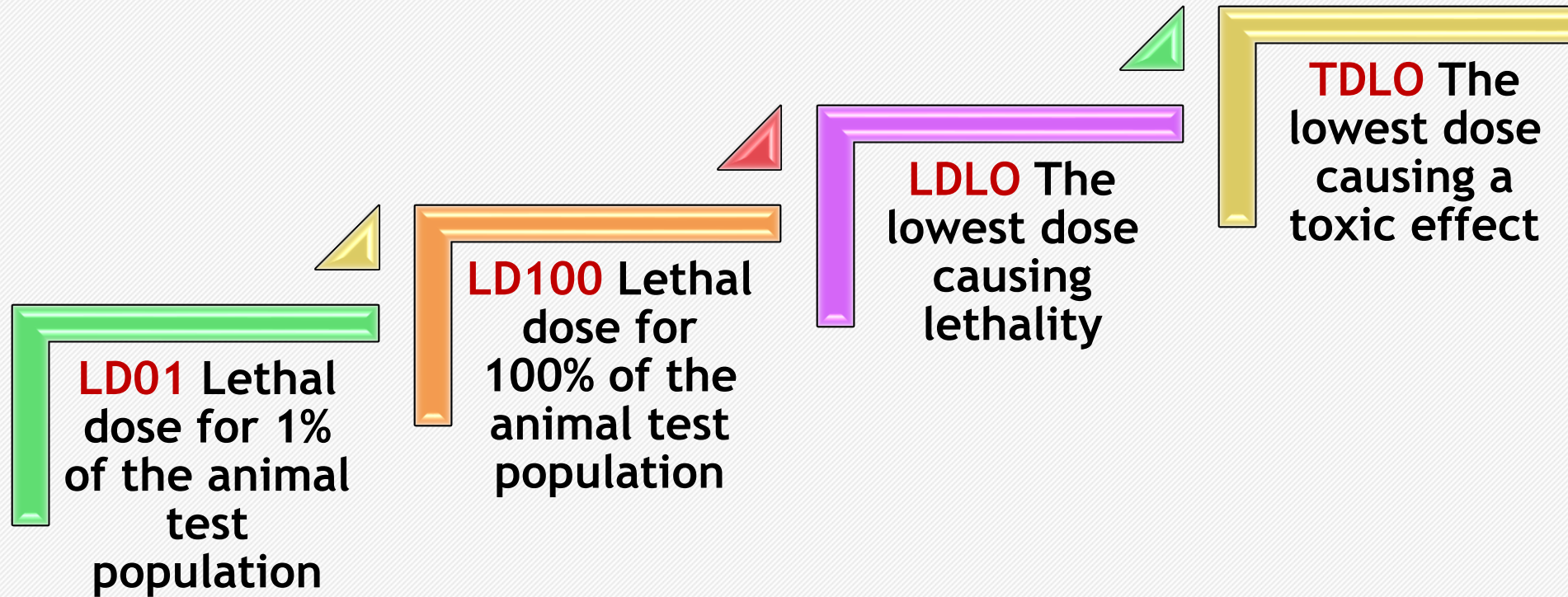
Factors effecting LD50

- **Results of LD50 study may affected by some factors which are:**
1. Species, Age, and Sex
2. Amount of food
3. Environment
4. Route of exposure (oral, dermal, inhalation)
5. Physical environment such as temperature and humidity

Importance of LD50

- Chemicals can have a wide range of effects on our health.

- Depending on how the chemical will be used, many kinds of toxicity tests may be required.

- Since different chemicals cause different toxic effects, comparing the toxicity of one with another is hard.

- To compare the toxic potency or intensity of different chemicals

Some other toxicity dose terms

LD01 Lethal dose for 1% of the animal test population

LD100 Lethal dose for 100% of the animal test population

LDLO The lowest dose causing lethality

TDLO The lowest dose causing a toxic effect

LD50 test features

1. **In nearly all cases, LD50 tests are performed using a pure form of the chemical. Mixtures are rarely studied.**

2. The chemical may be given to the animals by mouth (oral); by applying on the skin (dermal); by injection at sites such as the blood veins (i.v.- intravenous), muscles (i.m. - intramuscular) or into the abdominal cavity (i.p. - intraperitoneal).

LD50 test features

3. The LD50 value obtained at the end of the experiment is identified as the LD50 (oral), LD50 (skin), LD50 (i.v.), etc., as appropriate.

LD50 study design

Usually performed on mice.

Tested chemical given in different doses for different groups of animals usually 5-10 mice of both sexes.

The number of animals in each group should be differ from the number of total groups. Why?

LD50 study design

- **For calculating LD50 by any one method:**

✓find out the least tolerated (smallest) dose (100% mortality) and most tolerated (highest) dose (0% mortality) by hit and trial method.

✓Once these two doses are determined, select at least 05 doses in between the least tolerated and most tolerated doses, and observe mortality due to these doses.

Dose range calculation

- At beginning a pilot study done on small numbers of mice to select dose range.

- During such study an ascending doses of tested chemical should b e given for example 10, 30, 50, 70, & 100mg.

- Another method is the up and down methods (staircase method) in which only 2 mice or rats are used as fellow:

1. E.g. 50 mg of chemical administered then both animals observed for 24 hr.

2. Then subsequent dose increased by factor 1.5 if tolerated or decreased by factor 0.7 if found to lethal.

Methods to calculate LD50

- There are various methods to calculate LD50 values; like:
    1. Arithmetical method like Karber method
    2. Graphical method, like Miller and Tainter method
    3. Statistical approach.

Arithmetical methods (Karbers methods)

- It is the simplest and crude method of LD50 calculation.
- Can be performed in small group of animals
- In this method the interval mean of number of dead in each group is used as well as the difference among doses.
- Results of larger than lethal dose and smaller than the minimum dose are excluded.
- The sum product is divided by the number of animals in each group, then is subtracted from the least lethal dose to obtain LD50

Arithmetical methods (karbers methods)

- **Example:**
- ✓**Acute toxicity study was done on fifty adult mice, that were adopted into 5 groups (n=10 in each group). The results of study were provides with table below.**
- ✓**From these findings calculate LD50 using Karber method.**

Dose mg/kg	No. of animals	Dead
64	10	0
71	10	2
81	10	4
90	10	9
100	10	10

Arithmetical methods (karbers methods)

Dose mg/kg	No. of animals	Dose differences A	Dead	Interval Mean B	Product A*B
64	10	-	0	-	-
71	10	7	2	1	7
81	10	10	4	3	30
90	10	9	9	6.5	58.5
100	10	10	10	9.5	95
					190.5

LD50 = least lethal dose-(product of A*B)/N

LD50 = 100-190.5/10

= 80.95 mg / kg

Graphical methods (MILLER AND TAINTER)

- **This method is more accurate than karbers methods**
- In this methods the observed values are converted into probits using probit table
- **Then the probits values are plotted against log dose**
- **The probit values are plotted against log-doses**
- Then dose corresponding to probit 5, i.e., 50%, is found out.

Graphical methods (MILLER AND TAINTER)

- **Example :**
- In our study for estimation of LD50 of Chemical A, 5 doses were given intraperitoneally to 5 groups of rats, N=10 in each group as in table below.
- Animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms.
- After 24 hours, the number of dead rats was counted in each group and percentage of mortality calculated.
- **How can you get LD50 via graphical methods?**

Group	Dose (mg/kg)	Log dose	% Dead
1	25	1.4	0
2	50	1.7	40
3	75	1.88	70
4	100	2	90
5	150	2.18	100

Graphical methods (MILLER AND TAINTER)

Step one:

- **Find the Corrected % formula for 0 and 100% mortality as fellow:**

1. **For 0% mortality**

   Corrected %=100 * (0.25/N)= 100* 0.25/10= 2.5

2. **For 100 mortality**

   Corrected %=100 * (N-0.25/N) = 100 * (9.75/10)

   = 100* 0.975=97.5

Group	Dose (mg/kg)	Log dose	% Dead	*Corrected %
1	25	1.4	0	2.5
2	50	1.7	40	40
3	75	1.88	70	70
4	100	2	90	90
5	150	2.18	100	97.5

Graphical methods (MILLER AND TAINTER)

Step two:

- Find the probit fore each Corrected % from the probits table below:

Table-2: Transformation of percentage mortalities to probits

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Graphical methods (MILLER AND TAINTER)

Step two:

- **Then get the final table**

Group	Dose (mg/kg)	Log dose	% Dead	*Corrected %	Probits
1	25	1.4	0	2.5	3.04
2	50	1.7	40	40	4.75
3	75	1.88	70	70	5.52
4	100	2	90	90	6.28
5	150	2.18	100	97.5	6.96

Graphical methods (MILLER AND TAINTER)

Step three:

- **The probit values are plotted against log-doses, then dose corresponding to probit 5, i.e., 50%, is found out**

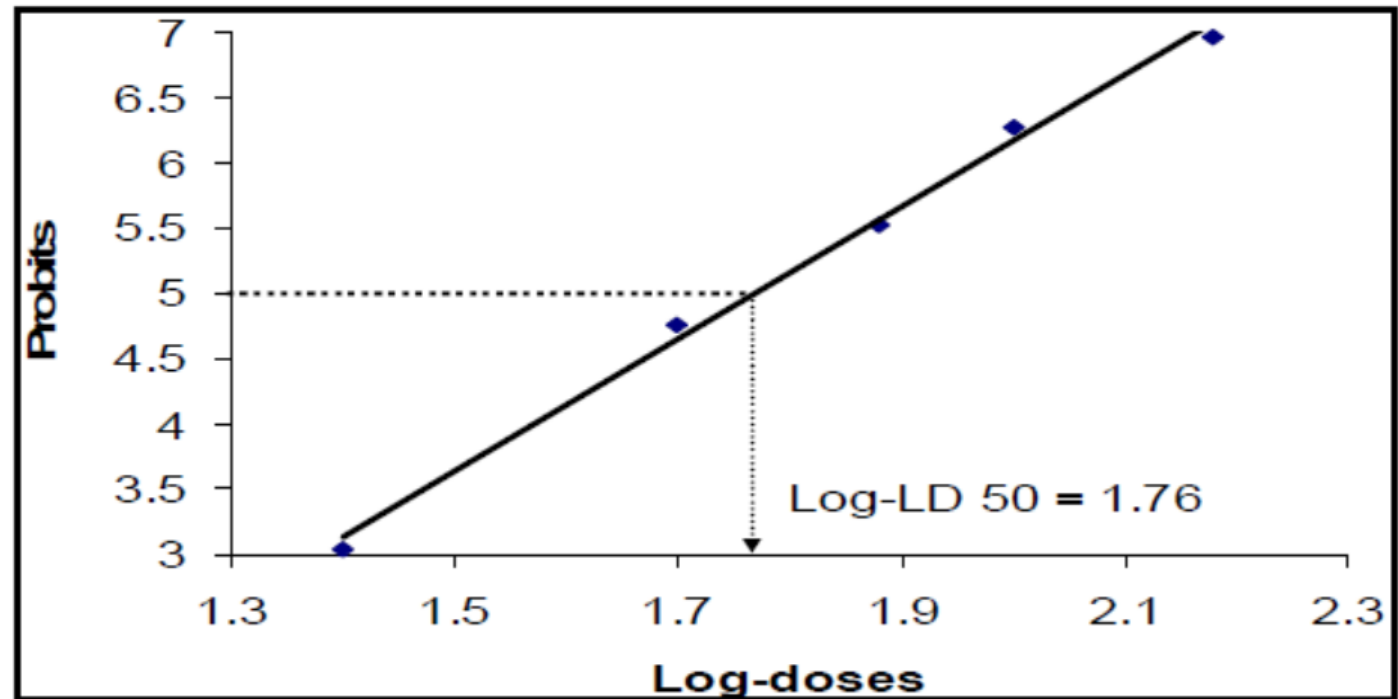- **In the present case the Log LD50 is 1.76 and LD50= 57.54 mg/kg.**

Figure-1: Plot of log-doses versus probits from

THANK YOU FOR YOUR ATTENTION